



Fig. 2.—Arthur Isaac Kendall, Ph. D., Director of Medical Research, Northwestern University Medical School, co-author with Royal Raymond Rife, Ph. D., of the paper on "Observations on *Bacillus Typhosus* in Its Filterable State."

turquoise-blue bodies in the uninoculated control K Media.

From the two facts thus far arrived at, namely, that the small, oval, turquoise-blue bodies were actively motile and also that they were cultivable from K Medium to K Medium, it is surmised that these small, oval, motile, turquoise-blue bodies are indeed the filterable forms of the *B. typhosus*.

There is another even more direct procedure for establishing the identity of these small, oval, motile, turquoise-blue bodies. It has been shown in previous communications<sup>3</sup> that agar cultures, or better, broth cultures of *B. typhosus* inoculated into K Medium, become filterable within eighteen hours' growth at 37 degrees centigrade. It should follow, inasmuch as not all of the bacilli appear to become filterable under these conditions, that at least some of the bacilli should have similar turquoise-blue granules within their substance if they are indeed passing to the filterable state. Also the free swimming filterable forms, the small, oval, motile, turquoise-blue bodies described above, should be simultaneously present.

Darkfield examination of such a culture eighteen hours old revealed unchanged, actively motile bacilli, bacilli with granules within their substance, and free swimming, actively motile granules. This culture examined in the Rife microscope with the quartz prism set at minus 4.8 degrees and with 5000 diameters magnification, showed very clearly the three types of organisms just described, namely:

First, unchanged bacilli: These were relatively long, actively motile, and almost devoid of color.

Second, long, actively motile bacilli, each with a rather prominent granule at one end. The granule in such an organism was turquoise blue, reminiscent in size, shape, and color of the small, oval, actively motile, turquoise-blue granules found in the protein medium (K Medium) where, it will be recalled, no formed (rod shaped) bacteria could be demonstrated. These bacilli having the turquoise-blue granules were colored only at the granule end, the remainder of the rod being nearly colorless, in this respect corresponding to the unchanged (nonfilterable) bacilli just mentioned.

Third, free swimming, small, oval, actively motile, turquoise-blue granules, precisely similar, apparently, in size, shape, and color to those seen in the granulated bacilli just described.

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From the fact that these small, oval, turquoise-blue bodies could be seen both in the parent rod and free swimming in the medium, it is assumed that these small, oval, actively motile, turquoise-blue bodies are indeed the filterable form of *B. typhosus*.

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#### REFERENCES

1. James A. Patten Lecture, Northwestern University Bulletin, Vol. 32, No. 5 (September 28), 1931.
2. Northwestern University Medical School Bulletin, Vol. 32, No. 8, (October 19), 1931, for full details.
3. Op. cit.

## TESTICULAR SUBSTANCE IMPLANTATION\*

### COMMENTS ON SOME SIX THOUSAND IMPLANTATIONS

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DISCUSSION by Jau Don Ball, M.D., San Francisco; Ralph A. Reynolds, M.D., San Francisco; H. Lisser, M.D., San Francisco.

RESEARCH relative to the transplantation of testes and implantation of testicular substance was begun at the California State Prison at San Quentin in 1918. It is now twelve years since this work was started. Approximately four thousand persons have been observed and have had administered to them some form of a gonadal product. It is now time to check up and evaluate, if possible, the results.

#### BROWN-SEQUARD'S OBSERVATIONS

Brown-Sequard, the father of endocrinology, in 1890 published the results obtained by injecting into himself an extract made by grinding in a mortar with water, and filtering, the testes of

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a young, vigorous dog. He claimed definite dynamic effects. At that time he was seventy-two years old and had many infirmities of old age: feebleness, rheumatism, decreased vigor, constipation, exhaustion, and inability to sleep. Following five injections of the extract he noticed that he tired less easily, regained much of his former strength, felt better physically, and was more alert mentally. "I can say, also, that my other forces—not lost, but decreased—have been remarkably strengthened."

After the publication of Brown-Sequard's findings, many physicians took up the procedure, but with them arose many charlatans and irregular practitioners. These latter played on the sex phase and made ridiculous claims. As a result, the better class of medical men were not impressed, and the matter lay semidormant until about 1912.

#### LYDSTON'S EXPERIMENTS

At this time Lydston conceived the idea of transplanting the testes. He chose himself to be the recipient. He procured the organs from a young man killed by accident. Lydston, like Brown-Sequard, was approaching old age and had some infirmities. His heart muscle was impaired, he tired easily, and he had a keratosis of his foot. After the transplantation these conditions were greatly improved. He was so impressed with the therapeutic value of transplantation of the testes that he wrote a monograph on the subject and published several reports in the medical journals.

#### THE SAN QUENTIN STUDIES

In 1918 the medical department of San Quentin undertook to confirm or disprove some of Lydston's assertions.

At the prison there is an average of three legal executions by hanging each year. Occasionally the bodies are not claimed and are available for postmortem study and research.

The first inmate patient, aged seventy-one, showed such an improvement in general health after the transplantation of a testicle that the operation was performed on twenty more. In none of these cases did the transplant live. It gradually became smaller and later disappeared. No attempt was made to do blood-vessel anastomosis, the organ being cut and sewed to the testicle of the recipient in such a way that the cortex of the two glands were approximated. Grafts of testes later removed and sectioned showed a necrosis of the tissues. Notwithstanding this necrosis, the patients received beneficial effects.

#### TRANSPLANTATION TECHNIQUE

Because of the scarcity of human material, it was determined to use that of rams and goats. In the early work a slice of ram's testicle the size of a silver dollar was placed in the pampiniform plexus. In other patients a similar piece was placed in a pocket made under the skin of the abdomen on either side of the navel.

With all these various techniques it was found that the material gradually absorbed, very seldom with suppuration.

In 1922 the technique was modified and has not been changed since. In this the scrotum of the ram is tied tightly above the testicles immediately after the death of the animal. The bag is then cut away and brought to the hospital. There the wool is clipped off and the skin painted with iodine. Under absolutely sterile conditions in the operating room the scrotum is incised and the testes removed. From these the tunica vaginalis propria is peeled off, leaving the matrix. This matrix is then placed in a small meat chopper and ground to about the consistency of tooth paste. To this is added a small amount of chloretone. This material is then placed in two-dram collapsible metal tubes with screw top. The whole tube is immersed in melted paraffin and placed in the refrigerator.

A metal syringe of two-dram capacity is used for administering this material. The plunger is removed and the contents of the tube squeezed into the breach of the syringe. A needle of 16-gauge and two inches long is attached.

In the meantime the patient is placed on the operating table, his abdomen is cleaned and iodine applied to either side midway between the umbilicus and the anterior superior spine of the ilium. At this same place a few minims of novocain, one-half per cent, is injected into the skin, making a small wheal two centimeters in diameter.

Into this wheal the larger needle is inserted and pushed underneath and parallel to the skin surface for one and one-half inches. The testicular material is then slowly injected as the needle is withdrawn, leaving a small burrow filled with the substance. The needle is turned at right angles and another injection made. In this way there are four burrows radiating from the center like the spokes of a wheel. These testicular substance implantations have been made about six thousand times in four thousand patients.

With this technique of testicular substance implantations there is very seldom any local reaction. The burrows can easily be felt by rubbing the finger over the abdomen. Occasionally redness and swelling develops and in about one per cent of the cases suppuration occurs. This quickly subsides when the site is opened. About ten patients in the whole series developed urticaria, and a like number had an edema of the scrotum and penis. This lasted only a few hours.

The procedure entails very little pain, as the novocain decreases this, and the subcutaneous fat is not highly sensitive.

#### COMMENT

The implantation of the whole testicular substance is considered an advantage over the mere injection of an extract. In fact, it is not definitely known just what part of the testes produces dynamic effects. This energizing part may be in the extract, or it may be in the residue, according to the method of extraction. With the implantation of the whole substance, on the other hand,

the body does its own extracting *in vivo*. It is known that nothing is left out.

When an extract is injected into the body, as adrenalin for instance, the effect is rapid but readily diminishes, for the extract is quickly absorbed. But with the implantation of the whole testicular substance, absorption is slow, and it seems reasonable to suppose that the system takes from the storehouse as a supply is needed. At any rate, the implants may be felt under the skin for weeks, and sometimes months. In other cases, however, it may be quite rapidly absorbed. Usually one treatment every two to three months is considered adequate. In the early work these implantations were given in many different cases regardless of the disease, in order to find out if possible just what conditions might be affected. In these trials it was felt that some patients were helped by this form of therapy.

*Acne Vulgaris.*—In the prison were a number of cases of acne vulgaris in boys and young men. One hundred and forty-eight patients with acne were treated. Ninety-nine of these were benefited in that their faces cleared up and showed marked improvement. Ordinarily this change took place within two months after the treatment.

*Diabetes.*—By accident it was found that in a diabetic patient the urinary sugar output became negative after an implantation of testicular substance.

Twenty-two other diabetics were then treated and it was found that in most of them the urinary sugar disappeared. The patients felt better, gained in weight, and were able to reduce their administration of insulin. From this experience it would appear that this form of therapy was a good adjuvant in the treatment of diabetes.

*Asthma.*—It was noticed that some of the patients who had asthma were benefited in that their attacks were less severe and less frequent. Perhaps no theories should be evolved, but it was felt that perhaps the implanted testicular substance had a stimulating effect on the adrenals in such a way that more adrenalin was sent into the system with a beneficial effect on the asthma.

*Constipation.*—In this connection it may be said that many of the patients reported that the constipation from which they suffered was markedly relieved by the treatment.

It is reasonable to theorize and say that here the increased adrenalin so affected unstriated muscles of the spastic bowels that normal movements took place. This phenomenon was recorded by Brown-Sequard fifty years ago.

*General Asthenia.*—The type of case which seemed to be most benefited was that of general asthenia, a condition where no definite pathology was found, but the patient felt tired, listless, and weary. Some of these symptoms had developed after influenza.

Of 429 men so classified, 370 patients received marked benefit. They gained in weight and activity and had a feeling of euphoria in contrast to their state of depression before.

*Sexual Function.*—Because of sex psychology considerable raillery has been directed toward this research, and because of it quacks and charlatans have arisen and better men have hesitated to accept deductions.

It is felt that in many cases any therapy which will increase one's feeling of well-being and step up his metabolism will at the same time increase his sexual powers. On the other hand, it has been demonstrated that patients with sexual lassitude, and even impotence, have had a return to normal after implantation of testicular substance.

In ten years of experimental work many interesting observations have been made.

In this work the usual technique of research has been reversed. Instead of experimenting with laboratory animals at first and finding out what effect testicular substance might have on them, work was done on human beings primarily and later checked up on animals.

For example: It has been seen that many men had unusual sexual manifestations after the implantation, and many who were sexually debilitated had a restoration of function. At the prison was a 14-year-old dog which had been a favorite with the guards, accompanying them on the night patrol. He gradually weakened from old age, was unable to make his rounds with the guards, and slept most of the time. General senility had set in. This old dog was secretly given an implantation of testicular substance. Within two days a decided change in his actions took place. He left his bed, resumed his walks with the guards, chased sticks, and for a time was greatly excited sexually. He even mounted his guard's leg in sexual embrace, as young dogs occasionally do. Psychology played no part in this.

*Urinary Sugar in Diabetes.*—Testicular substance implantations had the effect of lowering the urinary sugar in many of the diabetic cases. In some cases the sugar disappeared within two weeks without other medication or change of diet. In corroboration of this it was found that goldfish in a 0.1 per cent dextrose solution utilized about 22 per cent of the sugar in thirty hours. If to the same solution 100 milligrams of testicular substance was added, the same fish used 78 per cent of sugar in thirty hours. This would seem to indicate that this substance has some effect on sugar utilization.

*Euphoria.*—From practical experience it has been found that men to whom testicular substance has been administered show greater activity, are quicker in their movements and have a feeling of euphoria.

#### OTHER OBSERVATIONS

*Goldfish Experiments.*—An apparatus was devised to record, on a smoked paper, the movements of goldfish. It was found that the goldfish increased their activity 400 per cent when fed on testicular substance rather than on ground shrimp meat.

Into each of two separate bowls were placed two goldfish. They were weighed each week. To

those in bowl A was fed weekly one-quarter their body weight of ground-up meat. To those in bowl B was fed weekly one-quarter their body weight of ground-up testicular substance. Those fed on the beef gained much more rapidly in weight than did those feeding on testicular substance.

After ten weeks the diet was reversed, "A" getting the gonads, and "B" the meat. Here there was a steep rise in the weights of "B," and a very gradual rise in "A."

Those fish fed on the testicular substance were much more active, continuously swimming about or searching for food, while those fed beef were much less active.

*Frequency of Treatments.*—At the prison, part of one afternoon is devoted to testicular substance implantations. One ram supplies sufficient material for about twenty treatments. There is always a waiting list of fifty to one hundred men anxious for the treatment. This is a fair argument for its effectiveness.

#### CONCLUSIONS

After twelve years of work with this form of therapy it may be said that it has definite dynamic effects, and has palliative qualities in several definite conditions.

San Quentin Prison, San Quentin.

#### DISCUSSION

JAU DON BALL, M. D. (450 Sutter Street, San Francisco).—Doctor Stanley's paper should give an impetus to the search for the principles underlying his observed results of gland implantations in six thousand men in San Quentin prison.

Why testicular substance implants, as administered by Doctor Stanley, indicate certain results in certain physical ailments is not clear.

In my opinion, it will remain for the biochemist working in the field of endocrinology to ultimately answer the "Why" question.

But Doctor Stanley's account of his six thousand testicular gland implantations, stated without any dogmatic claims and with full recognition that the method was empirical, is, nevertheless, a very significant contribution to the study of the glands of internal secretion.

Over a period of about six years I have worked with Doctor Stanley on selected cases outside of prison. In a study of more than two hundred of these extramural cases, male and female, to whom more than four hundred implants of testicular substance were administered, we noted that 60 per cent responded favorably in varying degree.

The disorders included artificial and natural menopause, diabetes, arthritis, asthma, and acne. In addition a number of patients treated were psychiatric cases, suffering from depression, senility, etc.

In all these cases, in addition to the testicular substance implant, other treatments were also used, but I believe it was clearly indicated the implant was a very valuable aid to the other treatment, for in more than half the cases improvement was definitely noted only after the implant was administered or repeated.

Testicular implant, in my opinion, is not a rejuvenator, and Doctor Stanley does not make such a claim for it. Although we do not yet know "why," it does frequently stimulate metabolism, increase the general activity of the patient, give him a feeling of well-being, and in certain of the above-mentioned disorders improve and sometimes apparently clear up the condition.

RALPH A. REYNOLDS, M. D. (490 Post Street, San Francisco).—I wish to comment briefly regarding two phases of Doctor Stanley's work which it has been my privilege to observe during the past year and a half.

Doctor Stanley refers to the effect of testicular injections on the disappearance of acne. It is a well known fact that acne most commonly makes its appearance during the adolescent stage—from fifteen to twenty years—or during the period when the growth hormones are most active. Confronted, as Doctor Stanley was, with severe cases of acne vulgaris, usually in young men, it occurred to him that there might be a decrease in the hormone of one or more glands concerned with growth. Quite frankly, as he states, he empirically gave whole gland testicular substance to several of the most severe cases and found that most of them improved after the first injection, and many cleared up entirely after repeated injections. With Doctor Stanley's collaboration, I attempted to analyze these cases in order to determine whether or not there is any scientific explanation for his results. The age in the cases analyzed ranged from eighteen to twenty-five years. Most of the patients were tall and slender, and gave the general appearance of adolescent youths. About 70 per cent of them had no body hair, had light beards, and had skin of fine texture; in other words, they gave evidence of delayed development of adult characteristics. Long bone measurements were made of each member of this group. We know that in the case of the adult with normal development we can expect to find the arm spread equaling the height, while the distance from the pubis to the floor is about one-half the height. In the group examined it was found that four out of five revealed measurements showing the long bones to have developed in greater proportion than the rest of the body—that is, the arm spread exceeded the height, and the distance from the pubis to the floor more than equaled half the height of the body. This is generally looked upon as indicating a deficiency in testicular secretion. This, perhaps, partly at least, may explain Doctor Stanley's results in the treatment of acne in these cases. Certainly his work gives us a valuable clue in a more comprehensive approach to this dermatologic problem.

Doctor Stanley has referred to certain results in the lowering of blood sugar in diabetics following the injection of testicular extract. It is by no means easy to find any satisfactory explanation as to why testicular injections will lower blood sugar in diabetes, but that this is the case there can be no doubt. I have attempted to analyze twenty of these cases and have found that in severe cases (that is, blood sugars from 250 to 400 and requiring from forty to fifty units of insulin daily) five to six testicular injections are followed by a decrease in the blood sugar from one-third to one-half, the insulin requirement decreasing proportionately. In the less severe cases, blood sugar dropped to 130-150, the urine became sugar free, and insulin could be completely discontinued. About 75 per cent of these patients gave a history of obesity as existing before the onset of diabetes, indicating a general disturbance in growth and metabolism.

If we were correct in assuming that testicular extract stimulated the pancreas to greater insulin production, then in cases of renal diabetes with low blood sugar and fainting attacks we should expect to find the symptoms aggravated by testicular injections. This was found to be true in one case of renal diabetes carefully observed. This patient entered the prison in May 1929 with complaint of thirst, polyuria and fainting attacks, a blood sugar of from 60 to 80 milligrams, and sugar in the urine. He was given during the next year and a half ten testicular injections, with the result that he had more frequent attacks of dizziness, fainting, and weakness. He finally entered the hospital in November 1930 with a blood sugar of 60 milligrams. The gland injections

were discontinued six months ago and the patient was allowed to return to work. The last blood sugar, in March 1931, was 87 milligrams, and the symptoms of fainting and dizziness were much improved.

I do not pretend to know by what mechanism the testicular extract exerts its influence on these two conditions, but that there are certain objective effects, there can be no doubt.

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H. LISSER, M.D. (384 Post Street, San Francisco). Doctor Stanley has presented his clinical observations concisely and has spared his readers speculative embellishment. For this he deserves credit; and also for devising a relatively simple procedure for implanting testicular material without the necessity of hospitalization.

Indeed it may be granted that Doctor Stanley's method of implanting whole unextracted ram's testicular substance constitutes, up to the present, the only means of obtaining any clinical benefit from administering male gonadal material. Commercial testicular extracts are notoriously inert.

However, it is to be hoped that several researches, which are being prosecuted in various parts of the world, will ere long prove successful in providing the profession with a proper potent extract, comparable to such standardized preparations as thyroid, insulin, parathormone, pituitrin, adrenalin, and theelin. Moore and Gallagher of Chicago have isolated a potent product, but as yet an embarrassingly huge amount of material is required for a still more embarrassing "yield." The same difficulties confront Hartman and Swingle, each of whom have obtained an unquestionably potent hormone from the adrenal cortex, but in such tiny amounts that it costs \$3000 to treat one case of Addison's disease for one year.

But even when this desired male sex hormone becomes available, its clinical indications will remain to be determined. It may have a wide, vague range of usefulness as a sort of tonic, as seems suggested by Doctor Stanley's experience, but whether we will be able to diagnose specific states of male hypogonadism is not as certain as one might anticipate. The characteristics of both pre- and postadolescent castrates (eunuchs) have been abundantly recorded, more particularly in the classical studies of Tandler and Gross. Likewise deficiency signs and symptoms have been described under the caption "Eunuchoidism," initially by Duckworth and Griffith, but examples of these indubitable syndromes are relatively rare, and specific male gonad substitution therapy has been signally ineffective.

Furthermore, although it is hazardous to indulge in predictions, it is altogether likely that those who will seize upon a truly potent male sex hormone as a cure for impotency will be doomed to disappointment, for the following reasons: the vast majority of such cases are of psychic origin, a few are due to urological disease, and remarkably few indeed to endocrine deficiency. And it is rather startling and disconcerting that some incontestable cases of male castrates are on record who, nevertheless, were still possessed of potential coeundi.

Doctor Stanley's observations on the improvement noted from testicular implantations in such conditions as acne, diabetes, asthma, and constipation are certainly interesting and worthy of record, but will hardly supplant the recognized therapy for these ailments.

Perhaps we may hope that future discoveries will enable us to recognize mild cases of male hypogonadism which will respond to a potent testicular hormone, just as mild cases of hypothyroidism are now benefited by thyroid substance, but it must be granted that nature has been very generous in providing two testicles, and that a little of one seems to suffice ordinarily.

## FIBROIDS AND OVARIAN CYSTS COMPLICATING PREGNANCY\*

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DISCUSSION by Edward N. Ewer, M.D., Oakland; John W. Sherrick, M.D., Oakland; Frank C. Ainley, M.D., Los Angeles.

PREGNANCY may complicate the treatment of tumors, and tumors may complicate the treatment of pregnancy. Evidence of these facts is suggested by the following list of cases in my service at the University of California, and exclusive of consultation: two pregnancies with cancer of the uterine cervix, one with cancer of the labia, four with cancer of the breast, one with primary cancer of the lung, two with carcinoma of the appendix, one with endothelioma of the pharynx, two with brain tumors, one with tumor of the spinal cord, two with tumors of the bladder, one with sarcoma of the thigh, many with tumors of the thyroid, forty with uterine myofibroma, and eight with ovarian cysts. The only tumors which seemed lacking at first sight were bony tumors of the true pelvis of which we have examples only in nonpregnant women.

Our interest in this paper centers in fibroids and in ovarian cysts.

### FIBROMYOMATA

These tumors are often seen with pregnancies. For the most part they are of trivial size and of little clinical importance. Rarely, however, they may constitute a serious threat to the patient's safety in event the tumor is large and undergoes degenerations which demand attention in pregnancy or if it blocks labor as in the rare cases in which the tumor lies in the bony pelvis.

In earlier times fibroids sometimes complicated labor in a terrible manner, largely because surgery was practically interdicted. No matter what the present-day physician does in the way of manipulation, his result cannot be worse than was uniformly obtained by the cesarean operation in earlier time.

Now the conditions of former times no longer obtain. Fibroids of large size are rarely seen in pregnancy. Surgeons remove them while they are quite small and rarely in pregnancy. Moreover, they usually perform hysterectomy. Cervical fibroids are known to be rare, possibly only five per cent of all cases, and yet they constitute the bulk of fibroids which jam in the pelvis. Surgery, moreover, now offers us the possibility of removing fairly safely the offending tumor during pregnancy, or the child at term with or without the tumor or the uterus in event the woman has come to term and the fibroid threatens a blocked labor. Fairly safely, I say, in comparison with the mortality of former days, since even at

\* From the Woman's Clinic, University of California Hospital, San Francisco.

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